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Changes in Plasma TIMP-1 Levels after Resection for Primary Colorectal Cancer

C. FREDERIKSEN¹, A.F. LOMHOLT¹, G.J. DAVIS², B.L. DOWELL², M.A. BLANKENSTEIN³, I.J. CHRISTENSEN⁴, N. BRÜNNER⁵ and H.J. NIELSEN¹

¹Department of Surgical Gastroenterology, Hvidovre University Hospital, Hvidovre, Denmark;

²Abbott Laboratories, Abbott Park Rd, Abbott Park, IL, U.S.A.;

³Department of Clinical Chemistry, VU Medical Center Amsterdam, The Netherlands;

⁴The Finsen Laboratory, Rigshospitalet, Copenhagen Biocenter, Copenhagen;

⁵Institute of Veterinary Pathobiology, Faculty of Life Sciences, University of Copenhagen, Frederiksberg, Denmark

Abstract. *Background: Increased plasma levels of tissue inhibitor of metalloproteinases (TIMP-1) are associated with poor outcome in colorectal cancer (CRC), however postoperative changes in plasma TIMP-1 levels after resections for CRC have not been thoroughly evaluated. Materials and Methods: Plasma samples were collected from 45 patients with primary CRC, preoperatively, 2 hours after surgery, and at days 1, 2, 7, 28, 45, 60, 75 and 90 after surgery. TIMP-1 and CEA levels were determined using the ARCHITECT® Immunoanalyzer. Results: Postoperatively, the mean (geometric) TIMP-1 level increased and had a maximum level at day 1 ($p < 0.0001$). The mean TIMP-1 level then declined to a level at day 90 similar to the mean preoperative level. Conclusion: A mean decline in plasma TIMP-1 levels was not observed within 90 days. However, individual significant reductions of plasma TIMP-1 levels did occur within 28-60 days postoperatively.*

For patients who have been curatively resected for primary colorectal cancer (CRC), current international guidelines recommend postoperative carcinoembryonic antigen (CEA) testing, clinical evaluation and colonoscopy (1, 2). Postoperative surveillance has been suggested to improve

survival (3, 4), however, optimal patient follow-up in CRC remains controversial and its effectiveness is still debated (5-9). A number of biological markers have been suggested as additional prognostic factors providing information independently of the existing grading systems.

Identification of biological markers capable of early detection of recurrent or metastatic disease and potentially able to select patients for adjuvant therapy is greatly needed and would improve the postoperative surveillance of CRC patients.

Tissue inhibitor of metalloproteinases 1 (TIMP-1) plays a critical role in normal physiological processes as well as pathological conditions of tissue remodelling (10). TIMP-1 has been shown to be involved in many different steps of tumour genesis, inhibition of apoptosis (11, 12), stimulation of cell growth (13, 14) and the regulation of angiogenesis (15). In addition, a great number of major clinical studies have reported a promising prognostic value of tumour tissue or plasma TIMP-1 measurements in human malignancies including colorectal cancer, with high TIMP-1 levels associated with shorter survival (16-23). Plasma TIMP-1 levels have been evaluated during and after major surgery for CRC and it has been demonstrated that increased preoperative TIMP-1 levels did not decrease within 30 days postoperatively, not even in curatively resected patients (24). However, other results demonstrated that plasma TIMP-1 levels obtained at a median of 7 months postoperatively were strongly associated with patient outcome, with shorter survival predicted by high levels of TIMP-1 (25).

To determine whether plasma TIMP-1 is a potential postoperative surveillance marker in CRC, an assessment of the postoperative time frame during which the sample should be collected must be performed. The aim of this study was to establish the time frame in which postoperative plasma samples should be collected in order to determine if a patient was curatively resected or not. Plasma TIMP-1 levels were compared to plasma levels of CEA.

Abbreviations: TIMP-1: Tissue inhibitor of metalloproteinases 1; CEA: carcinoembryonic antigen; MMP: matrix metalloproteinases; CRC: colorectal cancer; TNM: tumour node metastasis; RI: reference interval.

Correspondence to: Camilla Frederiksen, MD, Department of Surgical Gastroenterology 435, Hvidovre Hospital, University of Copenhagen, Kettegaard Allé 30, DK-2650 Hvidovre, Denmark. Tel: +45 36322249, Fax: +45 36323760, e-mail: frederiksen@dadlnet.dk

Key Words: CEA, TIMP-1, lead-time, immunoassay, biological marker, postoperative follow-up.

Patients and Methods

Patients. In a study approved by the Danish Central National Ethics Committee, plasma sample sets were prospectively collected between June 2005 and June 2006 from 82 patients who underwent resection for primary CRC. Patients who received neoadjuvant, adjuvant or palliative radio- and/or chemotherapy (within 90 days) were excluded. The tumour stage was confirmed by histological examination of the resected tissue. Clinical stage and pathological features of primary tumours were defined using the TNM system and converted to stage I-IV (26). After surgical resection, patients underwent standard treatment and follow-up measures according to recommended guidelines. At the end of the study, all patient files were reviewed to detect any local or distant recurrence as well as any complications to surgery.

Blood sampling. Peripheral blood samples were obtained with informed consent from all individuals in agreement with the Helsinki II Declaration. Blood was drawn preoperatively, 2 hours after operation and systematically on postoperative days 1, 2, 7, 28, 45, 60, 75 and 90. The samples were collected into silicone-coated endotoxin-free tubes with EDTA as an anticoagulant (Vacutainers®; Becton-Dickinson, Mountain View, CA, USA). The sampling, handling, processing and storage of specimens were performed according to predefined Standard Operating Procedures previously described (27).

Laboratory methods. Plasma levels of TIMP-1 and CEA were measured with the Abbott ARCHITECT® i2000 automated immunoassay utilizing chemiluminescent magnetic particle immunoassay technology (28). Assays were run at the Abbott Center of Excellence research laboratory in Amsterdam, the Netherlands. Abbott in-house research prototype TIMP-1 reagents (29) and commercial ARCHITECT CEA (30) reagents were used for these studies. The interassay coefficient of variation (CV) for this study ranged from 3% to 6%. The intraassay CV ranged from 2% to 3%. Additional information concerning the ARCHITECT® i2000 is thoroughly addressed by Davis *et al.* (29).

Statistical analysis. The statistical analysis of TIMP-1 was performed using a linear model with repeated measurements with TIMP-1 on the log scale, assuming a normal distribution. The analysis was performed for each stage separately. The change in plasma TIMP-1 level compared to the preoperative level for individual patients was evaluated using a standard error estimated from 23 healthy individuals (31). From that study, the intra-individual variance was estimated and a comparison of a single follow-up measurement to the preoperative level taken in a short span of time was considered to be significantly lower if the TIMP-1 levels were reduced by 23% (one-sided). The same study also provided an estimation of the mean (geometric) TIMP-1 level for healthy individuals and the 95% reference interval (RI) for a single individual. The mean level was 82.0 ng/ml with RI from 59.4 ng/ml to 113.7 ng/ml. TIMP-1 levels above the upper limit of 113.7 ng/ml were considered elevated. Although we have previously shown that TIMP-1 is age dependent, it was not possible to make an age correction in the present study due to the age range of the healthy individuals.

CEA levels higher than 5.0 ng/ml were defined as elevated. *P*-values less than 5% were considered significant. All statistical calculations were made using SAS (Statistical Analysis System, version 9.1; SAS Institute, Cary, NC, USA).

Results

In total, 45 patients were available for statistical analyses after implementation of the inclusion and exclusion criteria. The baseline characteristics of the patients included in the study are shown in Table I. Not all patients were available for every sampling time point. In total, a median of 10 samples from each patient was collected (range 7-10 samples), with a median of 90 days (range 45-90 days) of follow-up.

TIMP-1. First the time course of pre- to postoperative mean TIMP-1 levels was evaluated. The preoperative mean levels (geometric means) of TIMP-1 stratified by stage are shown in Table II. Table III shows TIMP-1 at significant time points during the postoperative follow-up as compared to the preoperative value. TIMP-1 levels above the upper limit of 113.7 ng/ml were considered elevated and a reduction in the TIMP-1 level by 23% was considered significant (31). The analysis was performed for each stage separately. Figure 1 depicts the TIMP-1 level (as a percentage, as a function of the initial preoperative TIMP-1 level) at day 90 for each patient with an available blood sample at day 90 and for each stage.

Stage I. Four patients had preoperative TIMP-1 levels within the predefined RI ranging from 59.4 ng/ml to 113.7 ng/ml. Of the remaining 4 patients with preoperatively elevated TIMP-1, one patient reached a TIMP-1 level within the predefined RI at day 28 and the TIMP-1 level further decreased from day 45 with a 27% lower level at day 60. Another patient with preoperative TIMP-1 of 161 ng/ml showed a 35% decrease at day 90. A third patient with preoperative TIMP-1 of 177 ng/ml had a 23% decrease, but did not reach a level below the upper RI limit within the study period. The fourth patient had elevated TIMP-1 levels throughout the study period.

Stage II. Individual assessment showed that seven patients had TIMP-1 levels within the predefined RI prior to operation, whereas 12 patients had preoperative TIMP-1 levels above the predefined upper limit of 113.7 ng/ml. Of these 12 patients, only one with elevated preoperative TIMP-1 of 132 ng/ml had a statistically significant reduction of 35% at day 90.

Stage III. Individual assessment of these patients showed that six patients had TIMP-1 levels within the predefined RI at the time of operation. None of the four patients having elevated TIMP-1 levels preoperatively demonstrated a significant decrease (>23%) at any point in time.

Stage IV. Individual evaluation showed that all stage IV patients had high levels (>113.7 ng/ml) of TIMP-1 preoperatively. None of these patients demonstrated a significant reduction in TIMP-1 to a level below the predefined upper RI. One patient had a statistically significant 53% reduction from the preoperative level (268 ng/ml) starting at day 45, which significantly decreased up to day 90.

Table I. Baseline characteristics of patients with CRC included in the study.

Characteristic	No. of patients
Total number of patients	45
Gender	
M	25
F	20
Age (years)	
Median	75
Range	34-90
Tumour location	
Colon	24
Rectum	21
Tumour stage	
I	8
II	19
III	10
IV	8

Another patient had a postoperative TIMP-1 level reduced by 21% starting at day 28, which remained at this lower but elevated level up to day 90. Four of the eight stage IV patients had TIMP-1 levels that at the end of the study exceeded the preoperative level.

CEA. A CEA level above the 5.0 ng/ml cut-off value was considered elevated (32). Table II shows the preoperative mean levels of CEA stratified by stage. CEA levels at significant time points during the postoperative follow-up period relative to preoperative levels are shown in Table III. Individual assessment of changes in CEA was not performed.

Stage I. Preoperatively, two patients had CEA levels above the cut-off point of 5.0 ng/ml. CEA levels in these two patients decreased below the cut-off point. However, at day 90, two other patients had a CEA above the cut-off point.

Stage II. Preoperatively, four of the nineteen patients had a CEA level above the cut-off point, but they all returned to levels below this at day 90.

Stage III. Preoperatively, none of the patients in stage III had levels above the cut-off point and remained below this throughout the study.

Stage IV. Six patients with preoperatively elevated CEA still had CEA levels above the cut-off point of 5.0 ng/ml at day 90.

Discussion

The potential value of plasma TIMP-1 as a tumour marker in CRC has been thoroughly investigated. Preoperative plasma TIMP-1 has been shown to be elevated in patients

Table II. The pre-operative levels of TIMP-1 (ng/ml) and CEA (ng/ml) stratified by stage (geometric mean).

Stage	N	Variable	Mean	Minimum	Maximum
I	8	TIMP-1 CEA	122.4 3.1	90.5 0.6	177.3 7.4
II	19	TIMP-1 CEA	120.8 2.7	77.9 0.5	166.2 10.4
III	10	TIMP-1 CEA	112.7 2.5	81.2 0.8	145.0 5.9
IV	8	TIMP-1 CEA	201.9 15.7	122.0 0.9	283.0 165.4

Table III. TIMP-1 and CEA levels at significant time points during the study as compared with their preoperative value, stratified by stage.

TIMP-1							
Stage	N	2 Hours	p-value	Day 1	p-value	Day 90	p-value
1	8	86	0.04	214	<0.0001	97	NS
2	19	94	0.37	187	<0.001	107	NS
3	10	106	NS	220	<0.0001	109	NS
4	8	91	NS	23	<0.0001	97	NS
CEA							
Stage	N	2 Hours	p-value	Day 1	p-value	Day 90	p-value
1	8	71	0.004	63	<0.0001	83	0.58
2	19	62	<0.0001	77	0.02	74	0.02
3	10	61	<0.0001	61	<0.0001	81	NS
4	8	73	NS	92	NS	31	0.04

NS, non-significant; p-values as compared to preoperative level.

with CRC (16, 18, 33, 34) and preoperative plasma TIMP-1 levels were subsequently shown to hold prognostic information: high levels were independently related to poor survival (18, 25, 35, 36).

Assessment of TIMP-1 after resection of the primary CRC has also been investigated. It was shown in samples collected a median 7 months after surgery that patients with high postoperative plasma TIMP-1 had a significantly shorter overall survival than patients with low plasma TIMP-1 (25). Sequential TIMP-1 levels have been determined pre-, intra- and postoperatively and have shown that the surgical trauma led to a statistically significant increase day 1 after surgery, followed by a stepwise decline to levels similar to preoperative levels on day 30 postoperatively (24). However, in this time frame, the plasma TIMP-1 levels did not reach a

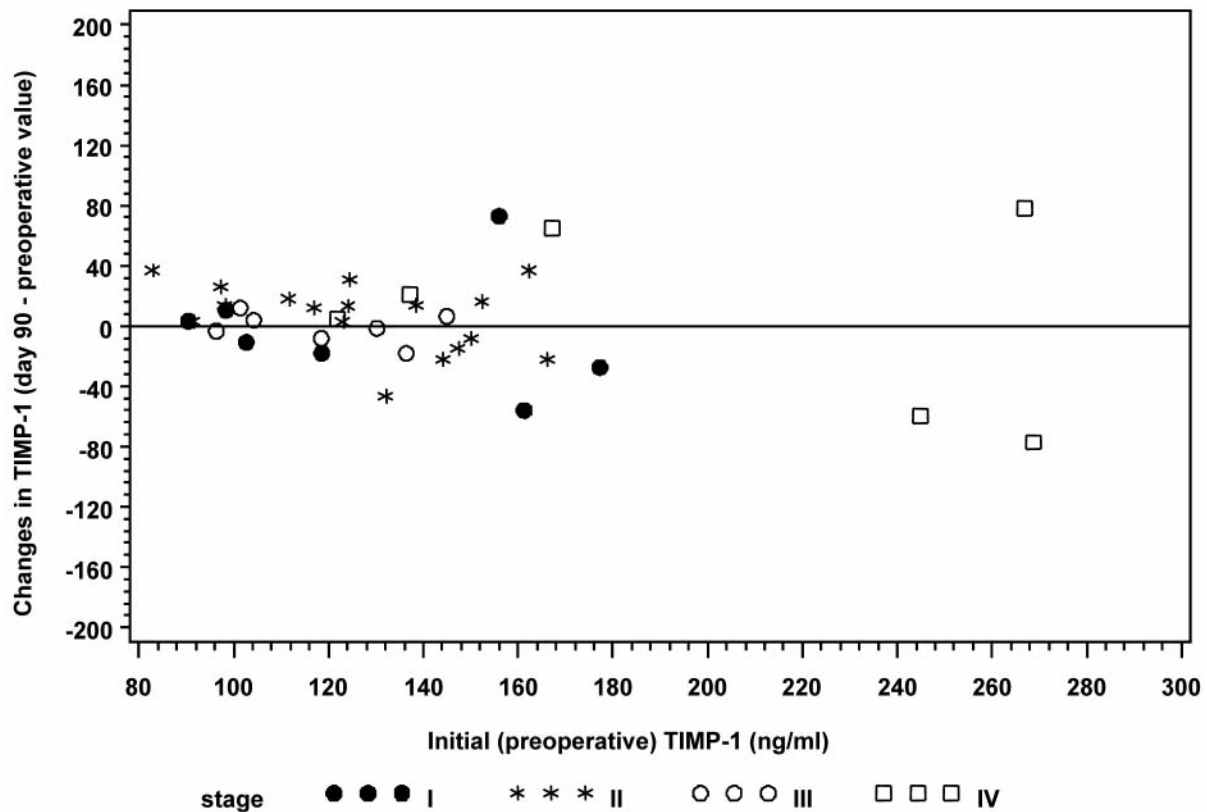


Figure 1. Depicts TIMP-1 levels at day 90 as a function of the initial TIMP-1 level for each patient and each stage.

level comparable to plasma TIMP-1 levels found in healthy individuals. It was suggested that a longer observation time was needed in order for TIMP-1 to decline to “normal” levels in patients who were cured by the primary surgery.

In the present study, pre- and postoperative plasma TIMP-1 levels were evaluated within an extended time frame of up to 3 months in patients resected for primary CRC. Changes in plasma TIMP-1 levels in peripheral blood were compared to changes in CEA levels. It was hypothesised that curative resection of CRC tumours would lead to a postoperative decrease in plasma TIMP-1 levels reaching the level of plasma TIMP-1 found in healthy individuals.

The results showed that preoperatively elevated TIMP-1 levels (above the 113.7 ng/ml) were found in all stages, although not in all patients. All patients with stage IV disease had elevated levels of plasma TIMP-1. Two hours postoperatively, mean plasma TIMP-1 levels demonstrated a small, non-significant decrease, which may be related to haemodilution. A significant maximum of mean plasma TIMP-1 was reached on day 1 postoperatively, independent of stage. This confirms previous results that TIMP-1 increases dramatically after major surgery (24). At present, the mechanisms leading to increased TIMP-1 levels

immediately after major surgery are not known in detail. Disintegration of platelets, neutrophils and macrophages due to the surgical trauma may play a role in elevation of postoperative TIMP-1 levels (37, 38). It is worth noticing that well-established inflammatory mediators such as interleukin-6 and C-reactive protein also show a postoperative response similar to that of TIMP-1 (39, 40).

Following the peak levels at day 1 after surgery, mean TIMP-1 levels declined gradually throughout the study period to levels on day 90 which were not significantly different from preoperative levels. There may be various explanations why we could not demonstrate a mean decline of TIMP-1 within 90 days to levels similar to those found in healthy individuals. The limited number of patients included with curatively resected disease may play a significant role. Only 45 out of 82 recruited patients were included in the final analysis and of these, only 37 underwent intended curative resection (stage I, II and III). We do not at present have information on recurrent disease in these patients, but these data will be obtained when available. Presence of micrometastatic disease in some of the intended curatively resected patients may possibly play a role in the observed limited decrease in postoperative mean TIMP-1 levels.

Additionally, prolonged recovery due to postoperative infectious and other complications may possibly also play a role in prolonged increase of plasma TIMP-1 levels. Wound healing is a complex process involving several phases including inflammation, proliferation and tissue remodelling, and matrix metalloproteinases (MMPs) and TIMPs are required in many of the steps of normal healing (41, 42). Previous reports have shown that remodelling after wound healing persists and is not normalized 6 months after surgery (43) and that differences in TIMP-1 levels postoperatively were associated with type and length of surgery, blood loss and postoperative complications (42). Thus, continuous wound healing with tissue remodelling may explain why TIMP-1 was not normalized after a follow-up of 90 days.

We considered whether individual patient assessment of TIMP-1 levels during the time of observation would provide additional information. Therefore, we evaluated changes in TIMP-1 levels for each patient during the study period. We used a change based on the variability of TIMP-1 estimated from healthy individuals (95% RI range 59.4 to 113.7 ng/ml). A reduction by 23% of the preoperative level was considered significant. Individual assessment of TIMP-1 levels showed that a number of patients with preoperatively high plasma TIMP-1 did demonstrate a significant reduction within the 90 days postoperatively. This significant reduction of more than 23% was evident by day 28-60 postoperatively and the optimal lead-time of postoperative sampling for plasma TIMP-1 measurements may therefore be within this time frame. It can be speculated that those patients who demonstrated a significant postoperative reduction in TIMP-1 could be those that were cured by the surgical intervention, while patients with no reduction or even an increase in plasma TIMP-1 postoperatively would be those that later developed clinically evident recurrent disease. This hypothesis is supported by a recent study in which it was shown that patients with elevated plasma TIMP-1 levels 7 months postoperatively had a highly significant risk of early death (25). A longer observation time will allow us to correlate the present plasma TIMP-1 findings with the course of the disease for the individual patients.

As CEA is the most extensively investigated and the only recommended marker for CRC surveillance, we included CEA measurements in this study. Current guidelines by ASCO (2) and EGTM (1) recommend testing of postoperative serum CEA every 3 months for at least 3 years in patients with stage II or III disease. In the present study, the mean CEA levels of patients with stage I, II and III declined immediately following surgery and continued to decline to day 90, with CEA levels being significantly lower than the preoperative CEA levels. Previous studies have shown that elevated CEA concentrations in CRC patients may persist for up to 5 months (44, 45). Only six out of the 37 patients in stage I, II and III had CEA levels above the cut-off point and of these only two had elevated

levels at day 90. Six out of eight patients in stage IV with preoperative CEA levels above the cut-off point remained elevated throughout the study. Thus, it is not obvious how monitoring of postoperative CEA measurements can guide clinicians as to whether a patient is cured by the primary surgery, while a further CEA increase postoperatively appears to be a valid marker of persistent disease or disease recurrence.

In conclusion, it was observed that preoperatively elevated levels of plasma TIMP-1 were present in all stages, although not in all patients with CRC. We did not observe a decline in mean TIMP-1 levels within the 90-day study. However, when assessing each patient individually, a number of patients with preoperatively high TIMP-1 did demonstrate a significant reduction within 28-60 days postoperatively. The optimal time point for postoperative plasma TIMP-1 sampling appears to be after day 60. Thus, it would be of interest to perform a clinical study where plasma specimens for TIMP-1 measurements were collected prior to treatment and then again following adjuvant chemotherapy to then determine if patients with persistent or even increasing TIMP-1 plasma levels were those who later developed recurrent disease.

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